10. (Amended) The method according to claim 1, wherein the hematological sample is a sample of peripheral blood, bone marrow fluid or urine of a mammal.

11. (Amended) The method according to claim 1, wherein in step (1), the leukocytic cells are fluorescence-stained after the erythrocytes are removed from the hematological sample.

REMARKS

I. New Declaration Required.

The Examiner required that Applicant submit a new declaration under 37 C.F.R. §1.67(a). (Office Action, p. 2). In making the objection, the Examiner contended that the date of signing by all Applicants is missing. (Id.) For the reasons set forth below, this rejection is traversed.

The Examiner is referred to MPEP §602.05 which states, in relevant part, "...the Office will no longer require a newly executed oath or declaration ...where the date of execution has been omitted." In view of the unambiguous statement in the MPEP that execution dates are "no longer required" for newly executed oaths or declarations, withdawal of the objection is respectfully requested. Applicants therefore submit that the requirement for an new declaration should be withdrawn.

II. Rejection under §112, first paragraph.

Claims 1-11 have been rejected under 35 U.S.C. §112, second paragraph. (Office Action, p. 2). The Examiner's statements in support of the rejection are discussed below.

Regarding the Examiner's rejection of claim 1 for improper antecedent basis, the claim has been amended in accordance with the Examiner's suggestions to change "in order to stain leucocytic cells" to read, "in order to stain the leucocytic cells," and to change "defining neutrophilic cells" to "defining the neutrophilic cells." In view of a grammatical irregularity, Claim 1 has also been amended to change "bonds" to "binds."

Claims 2-11 have been amended in accordance with the Examiner's suggestion to address the Examiner's objection of improper antecedent basis. In each claim, "A method," has been amended to "The method."

Claim 3 has been amended in accordance with the Examiner's suggestions to address the Examiner's objection of improper antecedent basis. In claim 3, "a group of all leucocytic cells." has been changed to "a group of all the leucocytic cells (or all the leucocytes)."

The Examiner has objected to claim 8 as including parenthetical symbols "because it is unclear whether the limitations within the parenthesees are part of the claimed invention." (Office Action, p. 3). Applicant respectfully points out that the parenthetical information in the claim are the abbreviations for the chemicals identified immediately before. Applicant believes it is clear from the claim that no additional limitations are included in the parentheses. It is further clear that such abbreviations would be recognized by those skilled in the art. See MPEP §2173.01. As such, Applicant respectfully traverses this requirement.

Claim 8 has been amended to correct the spelling of "allophycocyanin" in line 13.

Claim 10 has been amended to delete "a sample collected from a mammal by apheresis" in line 21. Support for the amendment can be found in the specification, for example, at page 7, lines 11-14.

Claim 11 has been amended in accordance with the Examiner's suggestions to address the Examiner's objection of improper antecedent basis. In claim 11, lines 23-24, "after erythrocytes are removed." is changed to read "after the erythrocytes are removed."

It is submitted that no new matter has been introduced by the foregoing amendments. Approval and entry of the amendments is respectfully solicited.

III. Rejections Under §102(b)

To reject a claim for "anticipation," the Examiner is required to establish "identity of invention." *Glaverbel Societe Anonyme v. Northlake Mktg. & Supply,* 33 USPQ2d 1496, 1498 (Fed. Cir. 1995). ***Each and every element*** recited in a claim must be found in a single prior art reference and arranged as in the claim. *In re Marshall*, 198 USPQ 344, 346 (CCPA 1978); *Lindemann Maschinenfabrik GMBH v. American Hoist and Derrick Co.,* 221 USPQ 481, 485 (Fed. Cir 1984). ***There must be no differences*** between what is claimed and what is disclosed in the applied reference. *In re Kalm*, 154 USPQ 10, 12 (CCPA 1967); *Scripps v. Genentech Inc.*, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991). "Moreover, it is incumbent upon the Examiner to ***identify wherein each and every facet*** of the claimed invention is disclosed in the applied reference." *Ex parte Levy*, 17 USPQ2d 1461, 1462 (BPAI 1990). And the Examiner is required to point to the disclosure in the reference "***by page and line***" upon which the claim allegedly reads. *Chiong v. Roland*, 17 USPQ2d 1541, 1543 (BPAI 1990). Applicants submit the Examiner has not satisfied this burden as to the references cited. Keeping in mind the above legal principles, Applicants turn to the cited references.

a. §102 (b) Rejection based on "Hubl"

Claims 1, 3-4 and 7-10 were rejected under 35 U.S.C. §102(b) as being anticipated by Hubl, et al., Cytometry (Communications in Clinical Cytometry (1997) 30:72-84 ("Hubl"). (Office Action, p. 4).

For the reasons set forth below, this rejection, respectfully, is traversed.

Hubl discloses a flow cytometric method for performing a five-part leukocyte differential based on three color staining. (Abstract) According to Hubl, the labeling procedure "allowed reliable identification of monocytes, basophils, and lymphocytes." (Page 75, Column 2, 3rd paragraph). Hubl also discloses light scatter characteristics and fluorescent antibody expression of eosinophils in samples, however, "*did not allow clear separation from mature neutrophils*." (Page 75, Column 2, 4th paragraph, emphasis added).

In making the rejection, the Examiner contended that Hubl teaches "a monoclonal antibody-based flow cytometric method of classifying and counting leucocytes by performing a five-part leucocyte differential using a three color staining procedure." (Office Action, p. 4). The Examiner further contended that Hubl teaches that "leukocytes are separated and identified based on the CD-45 expression and that basophil and eosinophil population can be separated by gating cells into the light side scatter and measuring CD-45 fluorescense intensity." (Office Action , p. 4).

As the Examiner will note, Hubl does not disclose the classification of immature granulocytes provided in step 5 of Applicant's claim. In fact, Hubl notes difficulties in separating eosinophils from mature neutrophils. (See, for instance, page 75, column 2, 4th paragraph).

In addition, the Examiner had not identified any disclosure in Hubl of a fluorescence based antibody which binds to at least one kind of immature granulocytic cell, as also recited in claim 1. Clearly, all of Applicant's claim limitations are not accounted for in the cited reference.

Because each and every element of claim 1 is not identified by the rejection to be present in Hubl, the rejection fails to set forth a prima facie case of anticipation. Accordingly, for the reasons set forth above, withdrawal of the rejection respectfully is requested.

b. §102(b) Rejection based on "Bowen"

Claims 1-10 were rejected under 35 U.S.C. §102(b) as anticipated by Bowen, et al., Laboratory Hematology (1997) 3:292-298 ("Bowen"). (Office Action, p. 4).

For the reasons set forth below, this rejection, respectfully is traversed.

Bowen discloses staining of prepared bone marrow specimens with three different monoclonal antibodies. (See p. 293, column 2, paragraph bridging p. 294, column 1). Specimens were then analyzed using flow cytometry. (Page 294, column 1, 2nd paragraph). Bowen then discloses that differential cell counts were done on five categories of granulocytes: (1) promyelocytes; (2) myelocytes; (3) metamyelocytes; (4) band and segmented neutrophils; and 5) mature and immature eonsinophils. (Page 294, column 2, first full paragraph).

In making the rejection, the Examiner contended that Bowen "teach abnormal patters of expression of CD16 and CD11b antigens by neutrophils in bone marrow of patients using flow cytometric monoclonal antibody-based, three color immunofluorescence technique which permits simultaneous characterization of different cell populations." (Office Action, p. 5).

Claim 1 requires "*defining* neutrophilic cells in the defined group of granulocyclic cells *different in degreee of maturity* on the basis of fluorescent intensities from a first and a second or third labeled antibody." (Emphasis added). Bowen does not describe that limitation as required by §102. To the contrary, Bowen appears to catergorize mature and immature cells together, rather than define and differentiate them. (Page 294, col. 2, first full paragraph). The Examiner's attempt to broadly encompass Applicants' specific limitations by stating that antibody binding in Bowen, "defines highly reproducible developmental maturation *patterns*" is simply not sufficient for a §102 rejection. (Office Action, p. 6, emphasis added). Aside from the fact that Bowen does not state this, "defining patterns" is not what claim 1 recites.

The Examiner also states that certain antibodies "are more useful in defining granulocytes in later maturation stages" than other antibodies. (Office Action, p. 6). Apart from being the Examiner's wording, not the description of Bowen, this fails to demonstrate where, if at all, Bowen discloses the step of defining the cells, as called for in the claim.

Moreover, Bowen does not disclose distinguishing mature eosinophils from immature granulocytes. Bowen clearly fails to disclose each and every claim limitation.

Because each and every element of claim 1 is not identified by the rejection to be present in Bowen, the rejection fails to set forth a prima facie case of anticipation. Accordingly, for the reasons set forth above, withdrawal of the rejection respectfully is requested.

c. 102(b) Rejection based on "Loken"

Claims 1-10 were also rejected under 35 U.S.C. §102(b) as being anticipated by Loken et al., EP 0317516, ("Loken"). (Office Action, p. 6).

For the reasons set forth below, this rejection, respectfully, is traversed.

In making the rejection, the Examiner contended that Loken discloses "a method and kit for classifying and counting lineages and stages of hematopoietic cells including leucocytes." (Office Action, p. 6). After a further discussion of the Loken disclosure, the Examiner concluded: "Loken discloses that by combining intensity of light scatter (FALS or SALS) and fluorescence intensity by different fluorochromes, various cell lineages and stages can be distinguished." (Office Action, p. 7).

Loken fails to disclose classifying a defined group of neutrophilic cells within a defined group of granulocytic cells into groups different in degree of maturity as is required in claim 1. Granulocytes in different maturity degrees are apparently not separated in Loken, nor are granulocytes distinguished from eosinophils. All claimed limitations, therefore, are demonstrably not present in Loken.

Because each and every element of claim 1 is not identified by the rejection to be present in Loken, the rejection fails to set forth a prima facie case of anticipation. Accordingly, for the reasons set forth above, withdrawal of the rejection respectfully is requested.

d. §102(b) Rejection based on "Jackson"

Claims 1,3 and 7-11 were rejected under 35 U.S.C. §102(b) as being anticipated by Jackson, et al., U.S. Patent No. 5,776,709 ("Jackson"). (Office Action, p. ???).

For the reasons set forth below, this rejection, respectfully, is traversed.

In making the rejection, the Examiner contended that Jackson discloses "a method for using flow cytometry for identifying and enumerating cells representing subpopulations of leucocytes in blood or bone marrow samples wherein a three parameter gate can be established

based on combined analysis of SALS, FALS, and fluroescence intensity." (Office Action, p. 7). The Examiner further contended that Jackson discloses incubating a sample with *at least* two fluorochrome labeled antibodies. (Office Action, p. 7).

Jackson does not disclose using three antibodies having the limitations recited by the claims and according to the process recited therein. Jackson clearly prefers two antibodies, a primary and secondary which may be the same or different. That Jackson leaves open the use of additional antibodies does not, ipso facto, disclose the facets of the claims. Application of a specific third fluorescence labeled antibody that binds to at least one kind of immature granulocytic cells is not disclosed in Jackson. Nor is there any disclosure in Jackson of a specific process using a third antibody different from the secondary and the primary antibodies, let alone the claimed process.

Similarly, there is no disclosure in Jackson regarding classification of cells different in degree of maturity. As this classification step is based on fluorescence from a second and a third antibody, Jackson, by referencing only primary and secondary antibodies, fails to disclose this.

Because each and every element of claim 1 is not identified by the rejection to be present in Jackson, the rejection fails to set forth a prima facie case of anticipation. Accordingly, for the reasons set forth above, withdrawal of the rejection respectfully is requested.

IV. Rejection Of Claim 11 Under §103 Based On "Hubl" or "Bowen" In View Of "McCarthy"

Claim 11 was rejected under 35 U.S.C. §103(a) as unpatentable over Hubl or Bowen in view of McCarthy, et al., Journal of Immunological Methods (1993) 163:155-160 ("McCarthy"). (Office Action, p. 8).

The disclosures of Hubl and Bowen have been discussed previously with respect to the respective rejections under §102(b).

McCarthy discloses a procedure for the quantitation by flow cytometry of function-associated antigens on neutrophils and monocytes in unlysed, unfixed, peripheral blood samples. (Abstract). McCarthy further discloses that Ficoll-Hypaque or dextran sedimentation can be used to purify peripheral blood neutrophils prior to labelling and flow cytometry. (Page 155, second column). McCarthy also discloses that such purification techniques "can by themselves induce changes in the expression of surface antigens." (Page 155, second column). McCarthy teaches that cooling blood samples to minimize meatabolic changes, then labelling and analyzing the samples promptly, may minimize opportunity for activation responses and chemically induced changes. (Page 156, column 1, paragraph 2). McCarthy discloses using this technique may avoid potential "artefacts induced by the use of fixatives, erythrocyte lysing agents or leucocyte preparation techniques." (Page 159, column 2, second paragraph).

In making the rejection, the Examiner apparently relied on Hubl or Bowen for "teaching" the particular process of cytometric analysis (it is not clear from the record).

The Examiner acknowledged that neither Hubl nor Brown teach a step wherein leukocytic cells are fluorescence-stained after erythrocytes are removed, as recited in Claim 11. (Office Action, p. 9).

To fill the acknowledged gap, the Examiner relied on McCarthy for teaching that "procedures of cellular separation or removal from other cellular populations are conventional and well-known in the art so that an issue of when such a purification or separation procedure is introduced into a method of flow cytometric analysis, i.e. before or after binding of a label to desired cells, is an obvious design choice." (Office Action, pp. 9-10).

The Examiner then contended that one of ordinary skill in the art at the time of the instant invention "would have reasonable expectation of success in separating and purifying leucocytes such as neutrophils from other cellular components such as erythrocytes using Ficoll-Hypaque and dextran sedimentation such as taught by McCarthy prior to labeling of leucocytes for cytometric analysis such as taught by Hubl and Bowen," (Office Action, p. 9) and "McCarthy specifically taught that such procedures of cellular separation or removal from other cellular populations are conventional and well-known in the art so that an issue of when such a purification or separation procedure is introduced into a method of flow cytometric analysis, i.e. before or after binding of a label to desired cells, is an obvious design choice." (Office Action, pp. 9-10).

With respect to Hubl or Bowen, Applicant reiterates that, at the very least, these fail to disclose, teach, or suggest the classification of granulocytes according to their maturity. Thus, for reasons already stated, Hubl and Bowen fail to account for all the elements of the base claim 1, from which claim 11 depends. As the Examiner acknowledges that Hubl and Bowen do

not disclose, teach or suggest the limitations of claim 11, the rejection must fail, before McCarthy is even considered.

To be sure, McCarthy does not teach removal of erythrocytes prior to fluorescence staining in the process it discloses (see abstract). It discloses "purifying blood neutrophils prior to labelling" (page 155, column 2) but points out the drawbacks of this in proposing an alternative procedure. (see paragraph bridging page 155, column 2 to page 156, column 1). As such, Applicant is unsure precisely why McCarthy is cited, and presumes by the tone of the Office Action that the Examiner has applied it merely to show the state of the art.

The drawbacks identified by McCarthy to the purification disclosed include that purification of blood neutrophils prior to labelling as they "induce changes in the expression of surface antigents." (Column 2, page 155.) Thus, the procedure McCarthy teaches is staining such that neutrophils, lymphocytes and monocytes are resolved while erythrocytes, which "stain very weakly," exhibit less fluorescent intensity (see abstract). McCarthy apparently teaches that it is not desirable to remove erythrocytes prior to staining. Moreover, McCarthy states "This procedure avoids potential artefacts that can occur due to the use of fixatives, erythrocyte lysing agents, or anticoagulants which are also divalent metal ion chelators." (Abstract).

Applicant, therefore, submits that McCarthy teaches away from removal of erythrocytes prior to staining and that available procedures affect the sample and are not desirable. McCarthy does not provide the requisite suggestion or motivation to do what the Applicant's have done. Thus, McCarthy fails to support the Examiner's rejection and does not fill the acknowledged gap within Hubl and Bowen, even if these references did disclose all elements of the underlying claim 1, which they do not.

At a minimum, to maintain a *prima facie* rejection based on obviousness, all elements and limitations of the claims must be present in the cited art. In order to establish a *prima facie* case of obviousness, i.e. the cited references must teach every element recited in the claims. In re Rouffet, 149 F. 3d 1350; 47 USPQ2d 1453 (Fed. Cir., 1998). All properties and attributes must be considered by the Examiner. In re Antonie, 195 USPQ6 (CCPA 1977).

Once each and every element recited in the claims is accounted for in the cited references, obviousness is then determined based on whether the art contains a teaching or a suggestion that one should deviate from the disclosure of a reference, which includes a teaching or suggestion which would have impelled one to do so and to do what the Applicants have done. Ex parte Markowitz, 143 USPQ 303, 305 (Bd. App. 1964). Applicant respectfully submits that the cited references fail not only to disclose or teach each element of the Applicant's claims, but also fail to provide the requisite suggestion *to do* what the Applicant has done. For these reasons alone, the rejection of the claims is insufficient as a matter of law. Ex parte Levengood, 28 USPQ2d 1300, 1301-02 (BPAI 1993).

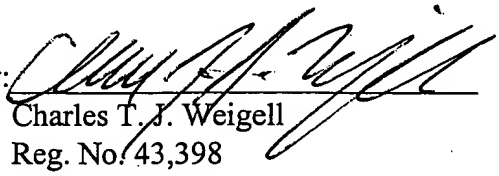
It is also well settled that an obviousness rejection must be based on facts, not generalities. Ex parte Saceman, 27 USPQ2d 1472, 1474 (BPAI 1993). "Cold hard facts." In re Freed, 165, USPQ 570, 571-72 (CCPA 1970). When a rejection under § 103 is not based on facts, it cannot stand. Ex parte Porter, 25 USPQ2d 1144, 1147 (BPAI 1992).

In sum, Hubl and Bowen are insufficient to account for all claimed limitations in combination, let alone those which the Examiner acknowledges are missing from these references. McCarthy not only does not disclose, teach or suggest the desirability of doing what Applicant's have claimed, it teaches away from what the Examiner regards as ordinary skill in

the art as applied to Applicants' claim. On all counts, the rejection conspicuously lacks support and should be withdrawn.

In view of the foregoing, favorable action on the merits, including entry of the amendments, withdrawal of each rejection and allowance of all claims, is respectfully solicited.

If the Examiner has any questions regarding this paper, please contact the undersigned attorney.

By: 

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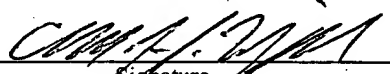
Name of applicant, assignee, or Registered Representative 
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What is claimed is:

1. A method for classifying and counting leukocytes comprising the steps of:

(1) adding to a hematological sample the following fluorescence-labeled antibodies labeled with fluorescent dyes which emit fluorescences distinguishable from each other;

(a) a first fluorescence-labeled antibody which binds [bonds] specifically to leukocytes,

(b) a second fluorescence-labeled antibody which binds [bonds] to at least one kind of neutrophilic cells, and

(c) a third fluorescence-labeled antibody which binds [bonds] to at least one kind of immature granulocytic cells,

in order to stain the leucocytic cells in the hematological sample, and

removing erythrocytes from the hematological sample;

(2) analyzing the resulting hematological sample using a flow cytometer to measure at least one scattered light signal and three separate fluorescence signals;

(3) defining a group of granulocytic cells on the basis of intensity of the scattered light and intensity of fluorescence from the first fluorescence-labeled antibody;

(4) defining the neutrophilic cells in the defined group of granulocylic cells on the basis of the intensity of the fluorescence from the first fluorescence-labeled antibody and intensity of fluorescence from the second or third fluorescence-labeled antibody;

(5) classifying the defined group of the neutrophilic cells into groups of neutrophilic cells different in degree of maturity on the basis of the intensity of the fluorescence from the second

fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody, and

counting the number of cells in each of the groups.

2. The [A] method according to claim 1, wherein in step (3), a group of all the leukocytic cells is defined and counted on the basis of the intensity of the scattered light and the intensity of the fluorescence from the first fluorescence-labeled antibody in addition to the group of granulocytic cells, and in step (5), the ratio of the number of the neutrophilic cells in each of the groups different in degree of maturity with respect to the number of all the leukocytic cells is calculated.

3. The [A] method according to claim 1, wherein the first fluorescence-labeled antibody comprises an anti-CD45 antibody.

4. The [A] method according to claim 1, wherein the second fluorescence-labeled antibody comprises an antibody selected from the group consisting of an anti-CD11b antibody, an anti-CD16 antibody, an anti-CD66b antibody and an anti-CD66c antibody, and the third fluorescence-labeled antibody comprises an antibody selected from the same group but different from the antibody of the second fluorescence-labeled antibody.

5. The [A] method according to claim 1, wherein the second and third fluorescence-labeled antibodies comprises any combination of an anti-CD16 antibody with an anti-CD11b antibody, an anti-CD16 antibody with an anti-CD66b antibody, an anti-CD16 antibody with an

anti-CD66c antibody, an anti-CD11b antibody with an anti-CD66b antibody, and an anti-CD11b antibody with an anti-CD66c antibody.

6. The [A] method according to claim 5, wherein the second and third fluorescence-labeled antibodies comprise the anti-CD16 antibody and the anti-CD11b antibody.

7. The [A] method according to claim 1, wherein the scattered light measured is side scattered light.

8. The [A] method according to claim 1, wherein the fluorescent dyes are selected from the group consisting of fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin [allophicocyanin] (APC), Texas Red, PE-CY5 and peridinin chlorophyll protein (PerCP).

9. The [A] method according to claim 7, wherein the fluorescent dyes of the first, second and third fluorescence-labeled antibodies for emitting distinguishable fluorescences comprise a combination of FITC, PE and PE-CY5 or a combination of FITC, PE and PerCP.

10. The [A] method according to claim 1, wherein the hematological sample is a sample of peripheral blood, bone marrow fluid or urine of a mammal. [or a sample collected from a mammal by apheresis].

11. The [A] method according to claim 1, wherein in step (1), the leukocytic cells are fluorescence-stained after the erythrocytes are removed from the hematological sample.